necessary for replication of said second origin of replication, wherein said first and second origins of replication may be the same or different.

REMARKS

Claims 26-54 were pending. Claims 26-38, 52, and 54 were indicated as allowable in the Office Action. Claims 39-51 and 53 were rejected. In view of the foregoing amendments and arguments that follow, Applicants respectfully request withdrawal of all rejections upon reconsideration.

Preliminarily, Applicants acknowledge with appreciation that the Examiner has not applied the new matter rejection against claims 26-53. Applicants also acknowledge with appreciation the Examiner's observation that Atweh does not specifically teach an LCR-containing plasmid having an origin of replication operative in mammalian cells.

35 USC §112 rejections

Claims 50 and 51 were rejected under 35 USC §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner directed Applicants to the reasons of record applied to claim 22 in Paper No. 5 (the Office Action mailed 7/9/99) and the Advisory Action mailed 11/1/02 (Paper No. 32). Applicants respectfully traverse this rejection

The Examiner raised two main arguments in support of this rejection in Paper No.5: 1. there are no *in vivo* exemplifications in the specification and gene transfer and expression from gene delivery vectors is highly unpredictable; and 2. the episomes would not be expected to be stably inherited because they do not have a centromere.

To address the former, Applicants enclose Giraldo and Montoliu, *Pigment Cell Res.*, 15:258-264, 2002. Giraldo and Montoliu demonstrate the benefits of using the appropriate LCR (in this case the tyrosinase LCR) in an artificial chromosome construct to obtain tissue-specific expression of a transgene *in vivo*. Although the authors seem to suggest that the construct becomes integrated, the reference is being relied upon primarily to support *in vivo* expression using constructs similar to those claimed.

Applicants also enclose Chow *et al* (Gene Therapy 9: 327–336, 2002). In Chow

et al, the authors report that their findings imply that expression of some regions within replicating episomal vectors (REVs) seems to be prone to silencing, but that the inclusion of the βLCR prevented repression of gene function in the REVs (p333, second column, middle of last paragraph). In fact, Chow *et al* asserts that "our results indicate for the first time the feasibility of using REVs (Replicating Episomal Vectors) for gene therapy of beta-thalassaemia and sickle cell disease..." (p333 last line to p334 line 1).

To address the latter, Applicants rely upon Chow *et al* and, further, Westphal *et al* (Human Gene Therapy 9: 1863–1873, 1998) (copy enclosed) and Simpson *et al* (Mol Cell Biol 16: 5117–5126, 1996) (copy enclosed). Chow *et al* show the use of episomal constructs carrying LCRs (beta-globin) to obtain appropriate expression of an operatively-linked transgene, and that such episomal constructs are persistent with or without the application of selective pressure in the form of drug selection (p333, column 2, paragraph 2).

Westphal *et al* demonstrate the use of an EBV Ori P-based human artificial episomal chromosome system for sustained expression of beta-globin transgenes. The authors state that such episomes have "a half-life of approximately 3 months in **actively dividing** human cells grown in the absence of hygromycin selection" (p1871, column 2, paragraph 2, emphasis added) and go on to comment thereafter

> [c]onsidering that most cells in the human body are non dividing or semiquiescent, such an efficiency of persistence may be sufficient, or may require medium-term repetitive treatment strategies... Alternatively, one could use in vivo-selectable resistant markers such as the methotrexate/DHFR system... Hence, a gene therapy strategy based on episomal genomic activation may become an attractive strategy for treating those diseases recalcitrant to long-term phenotypic correction via gene expression".

Simpson *et al* make clear that vectors based on yeast artificial chromosomes can be stably maintained as extrachromosomal molecules in human cells, even without externally applied selective pressure. They state (p5117, column 2, paragraph 2, lines 8–11) "...OriPACs of up to 660 kb in size are maintained as unrearranged, episomal

molecules for long periods of time in the absence of selection, and stable maintenance appears to be due to the association of the episomes with host cell chromosomes."

It is respectfully submitted that the disclosures of the application, together with the background art and knowledge of an appropriately-skilled person in the field are sufficient to enable use of the invention for *in vivo* applications.

Claim 39, and the claims dependent therefrom, i.e., claims 40-49, 51, and 53 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite in view of the recitation of "i)" twice in claim 39. Claim 39 has been amended herein to remove the inadvertent second recitation of "i)." Applicants submit that this rejection has been obviated by amendment.

Applicants respectfully submit that all claims are in condition for allowance and respectfully request early notification of the same.

Respectfully submitted,

Date: July 25, 2003

Doreen Yatko Trujillo
Registration No. **35,719**

COZEN O'CONNOR PC
1900 Market Street, 6th Floor
Philadelphia, PA 19103-3508
(215) 665-5593 – Telephone
(215) 701-2005 – Facsimile